
Raman diagnosis of nucleic acid structure: sugar-puckering and glycosidic conformation in the guanosine moiety

Yoshifumi Nishimura*, Masamichi Tsuboi*, Toru Nakano⁺, Shigesada Higuchi⁺, Tomohiro Sato[§], Toshio Shida^{**}, Seiichi Uesugi^{**}, Eiko Ohtsuka^{**} and Morio Ikehara

*Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, and ⁺Mitsubishi-Kasei Institute of Life Science, 11 Minamiooya, Machida, Tokyo, Japan

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ABSTRACT

Observations of Raman spectra of various nucleic acids indicate that the guanine ring breathing frequency is sensitive to the internal rotation angle around the glycosidic bond and to the conformation of the five-membered ring of the ribose residue that is directly connected with the guanine residue in question. It is found that 682 cm⁻¹ for C2'-endo-anti, at 665 cm⁻¹ for C3'-endo-anti, and at 625 cm⁻¹ for C3'-endo-syn. A DNA octamer (GpGpApApTpTpCpC) shows, in its aqueous solution, a broad Raman band at 680 cm⁻¹ with a tail at 670 cm⁻¹. This fact suggests that the guanosine residues in this oligomer take primarily C2'-endo-anti conformation but an appreciable amount of fluctuation of the ribose ring structure towards C3'-endo is involved.

INTRODUCTION

The DNA double-helix is now found to be more flexible¹⁾ than we once imagined to be. Its conformation varies depending upon the nucleotide sequence and the molecular environment. Therefore, it is now required to determine a variety of its local structures for various particular nucleotide sequences and in various molecular environments (various ionic strengths and/or various ethanol contents in its aqueous solvents, for example). Raman spectroscopy can meet such a requirement because of the following advantages: (i) it can be applicable both for crystals (for which X-ray structural analyses have been made) and aqueous solutions, (ii) it presents a great amount of information through its great number of Raman bands, (iii) it gives pieces of information for every conformer separately (instead of giving an average type of information), and (iv) it requires only a small amount of sample.

In view of this situation, we have started a detailed

study of the correlation between the Raman spectra and conformations of polynucleotides. In this paper, some of the results on the local conformation of the guanosine moiety will be described. A few examples are also presented showing how to interpret the Raman spectrum of a DNA oligomer with respect to its conformation.

EXPERIMENTAL

The crystal of calcium guanylyl-3', 5'-cytidine (GpC) was prepared as described by Hingerty et al.²⁾ The crystal structure was determined by X-ray diffraction analysis³⁾, and the same crystal (powder) was subjected to our Raman spectroscopic study. Yeast killer RNA was prepared according to the method of H. M. Fried et al.⁴⁾ and then the L-ds RNA was purified by preparative agarose slab gel electrophoresis. This is known to have fully double-helical structure⁴⁾. A DNA-RNA hybrid was prepared by the use of *Escherichia coli* RNA-polymerase with ϕ X174 phage DNA as the template. The details of the procedure will be given elsewhere⁵⁾. Hexadeoxyribonucleotide d(CpGpCpGpCpG) was prepared as described by Uesugi et al.⁶⁾ Another DNA oligomer (Octamer) d(GpGpApApTpTpCpC) was obtained by a method given by Ohtsuka et al.⁷⁾

Raman spectra were observed by the use of a JEOL (Japan Electron Optics Laboratory, Company) JRS-U1 spectrophotometer. Some of the spectra were also observed by the use of a Jasco R-800 spectrophotometer with a data processor. The exciting line 514.5 nm of an argon ion laser (NEC model GLS 3300) was used. The power was 300 mW at the sample point.

Part of the observed Raman spectra are shown in Figure 1, along with the Raman spectra of DNA observed by Martin and Wartell⁸⁾ and the Raman spectrum of Poly(dG-dC) observed by Thamann et al.⁹⁾ for comparison. Some of the observed Raman bands are listed in Table 1, with proposed assignments. The Raman spectra of the two DNA oligomers are shown in Figure 2 and partly with a higher resolution in Figure 3.

ASSIGNMENTS OF THE RAMAN BANDS

In the present study, our attention was focused into the spectral region 850-500 cm^{-1} , where a number of Raman bands are found to be potentially useful in a diagnosis of the nucleic acid structure as shown in Table 1.

810 and 835 cm^{-1} . The Raman band in this frequency region was assigned by Erfurth et al.^{10),11)} to a sugar-phosphate backbone vibration, which is sensitive to whether its conformation is A-form (at 810 cm^{-1}) or B-form (at 835 cm^{-1}). This

Table 1. Assignments of some of the observed Raman bands (cm^{-1})

GpC	d(CG) ₃ ^{b)}	d(CG) ₃	DNA ^{c)}	DNA ^{c)}	DNA	dsRNA	DNA-RNA	Assignment
Crystal	in H ₂ O, low salt	in H ₂ O, low salt	in H ₂ O + EtOH ² (85%)	in H ₂ O + EtOH (60%)	in H ₂ O, low salt	in H ₂ O	in H ₂ O	Form
A form	Z form	B form	A form	B form	B form	A form	A form	Source
810	(810) ^{a)}	828	809	835	835	814	813	Rp ^{d)} B A
785	785	785	(794) 785 (765)	(790) 785	(790) 785	785 (773)	785	thymine cytosine
(748) (735)	(737)		(751) 731	751 731	752 730	755 725	748 725	{Thymine Adenine or RP
714			709			712	(710)	RP A B
668	(675)	682		682 (672)	683 (672)			Guanine A
(625)	625	640	665 (645) (624)	645	645	668 (627)	668	Guanine Z

a) Those in parentheses were observed as shoulders, or as very weak peaks.
 b) Reference 9)
 c) Reference 8)
 d) RP = Ribose-phosphate main chain.

assignment was supported by our present study, as well as by many other studies including recent one by Thomas and Peticolas 12). GpC crystal, which is known to have the A-form gives a band at 810 cm^{-1} , and yeast-killer double-helical RNA (A-form) at 814 cm^{-1} .

795 and 785 cm^{-1} . A Raman band assignable to the ring-breathing vibration, in which all of the nine skeletal bonds stretch in phase, of cytosine is expected at about 785 cm^{-1} 13), 14). All of the nucleic acids examined in the present study have cytosine residues, and all give strong Raman band at 785 cm^{-1} (see Figures 1 and 2, and Table 1). This does not seem to be structure-sensitive. The ring-breathing vibration of the thymine residue is considered to be located at 795 cm^{-1} . It was observed as a small peak or as a shoulder for double-helical DNA (see Figure 1, (d) and (g) 8). In DNA-RNA hybrid, this seems to be hidden by nearby stronger two peaks. Thus, the trough at 795

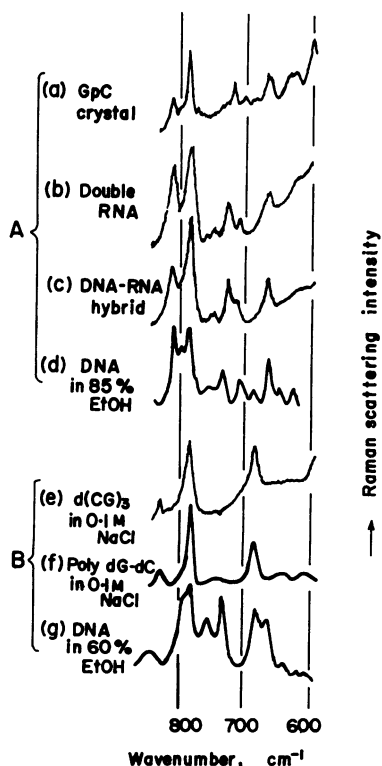


Figure 1. Raman spectra of nucleic acids in the $850\text{--}600\text{ cm}^{-1}$ region. (a), (b), (c), and (e): Reproductions of the curves obtained in the present study. (d) and (g): Schematic reproductions of curves given in reference (10). (f) Schematic reproduction of a curve given in reference (11).

cm^{-1} is much shallower for DNA-RNA hybrid (Figure 1 (c)) than for double-helical RNA (Figure 1 (b)). It should be pointed out here that a Raman line at 795 cm^{-1} was once assigned to an O-P-O single bond stretching vibration of the B-form DNA mainchain¹¹). In the Raman spectrum of B-form $\text{d}(\text{CG})_3$, however, no 795 cm^{-1} line is apparent besides its strong 786 cm^{-1} line (see Figure 3, lower).

750 and 730 cm^{-1} . Both of these strong Raman bands are found only when the nucleic acid in question has the adenine-thymine base pairs. In the Z-form crystal of $\text{d}(\text{CG})_3$ examined by Thamann et al⁹), the two weak bands observed at 748 and 735 cm^{-1} cannot be caused by the A.T pair, they are probably caused by mainchain vibrations characteristic of Z-form.

712 cm^{-1} . A weak band is observed at 712 cm^{-1} for the A-form nucleic acid, irrespective of its base composition. This is assignable to a mainchain vibration characteristic of the A-form.

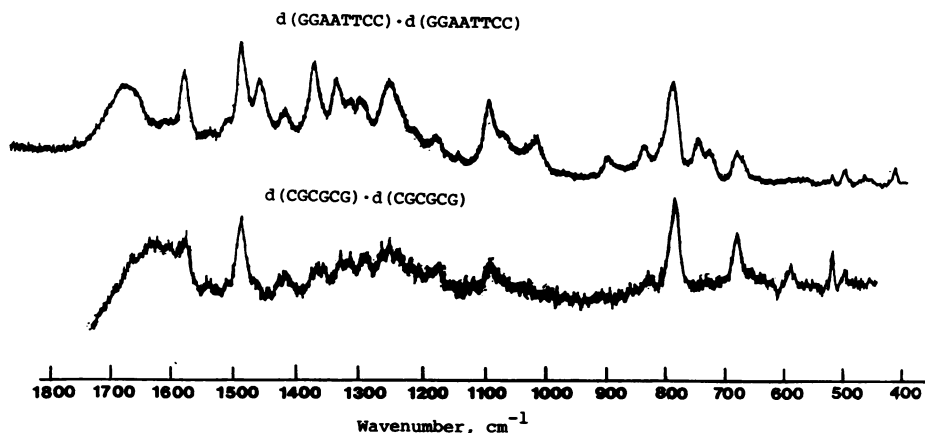


Figure 2. Raman spectra of a duplex of an octadeoxyribonucleotide $\text{d}(\text{GGAATTCC})$ (upper) and another duplex of a hexadeoxyribonucleotide $\text{d}(\text{CGCGCG})$ (lower) in their aqueous solutions. Solvent: 0.15 M NaCl , pH 7.0 . Concentration: 5% . Temperature: 20°C . Excitation: 514.5 nm (Ar^+ laser). A sharp peak at 521 cm^{-1} is caused by the natural emission from the Ar^+ discharge tube, and was used as one of the internal standards for the frequency calibration. Spectral slit width was set at 8 cm^{-1} .

682 and 665 cm^{-1} . These are considered to correspond to the 650 cm^{-1} strong band of guanine itself. Pohl et al.¹⁵⁾ once showed that the corresponding Raman line of poly (dG-dC) is located at 682 cm^{-1} when it is in a low salt solvent, and at 625 cm^{-1} in a high salt solvent. Recently, Thamann et al.⁹⁾ showed that the guanine line at 682 cm^{-1} is assignable to B-form DNA and that at 625 cm^{-1} to Z-form DNA. We have now inquired how about A-form, and chosen samples which are surely in the A-form. The Ca-salt crystal of GpC is known by an X-ray crystallographic study^{2), 3)} to have "A-form". Also, double-helical RNA is generally known to have A-form. In addition, calf thymus DNA in 85% ethanol (plus H_2O) was found to have A-form, judged from its characteristic Raman band at 808 cm^{-1} ⁸⁾. All of these, as shown in Figure 1, gave strong Raman band in the range of 670 ~ 665 cm^{-1} , at slightly lower frequency than the guanine band for B-form, but still considerably higher frequency than for Z-form.

625 cm^{-1} . According to Thamann et al.¹⁾, this is the frequency, where guanine in Z-form nucleic acid gives a strong Raman band. One must be careful, however, because a weak Raman band is found also at 625 cm^{-1} , for A-form nucleic acid.

SUGAR PUCKERING AND BASE FREQUENCY

In the course of our Raman spectroscopic studies of nucleic acids, we noticed that some of the Raman frequencies, primarily assignable to the base-residue vibrations, depend on the mainchain conformation. A good example is the guanine band around 650 cm^{-1} ; it has been shown that this is located at 665 cm^{-1} for A-form, 682 cm^{-1} for B-form, and 625 cm^{-1} for Z-form. We propose to consider that such a frequency difference is caused by the difference in the local conformation of nucleoside rather than by the difference in the whole mainchain conformation. According to our recent normal coordinate analysis with a set of force constants derived by an ab initio MO method, the 650 cm^{-1} band is assignable to a ring breathing vibration of the guanine ring. This is nearly localized in the base moiety, but its glycosidic bond ($\text{N}-\text{C}_1'$) stretching takes place with a great amplitude in the vibration. Therefore, an

appreciable vibrational coupling should occur between the guanine vibration in question and some of the ribose vibration. Thus, the frequency of the strong Raman band in the 650 cm^{-1} region may vary depending upon whether the glycosidic linkage is syn or anti primarily, and secondarily depending upon whether the ribose is C2'-endo or C3'-endo. This dependence is summarized in Table 2. This table should in future be refined and expanded, because the glycosidic linkage region does not necessarily take only two conformations, syn and anti, but can have whole variety of the internal rotation angle χ from 0° to 360° . Also a ribose ring can take not only C3'-endo and C2'-endo conformations, but also any other alternative of pseudo-rotation angle θ of five-membered ring¹⁶⁾, where $\theta = 0 \sim 360^\circ$.

DISCUSSIONS ON THE INDIVIDUAL STRUCTURES

DNA-RNA hybrid (see Figure 1 (c)). A strong Raman band at 813 cm^{-1} and a weak shoulder at 712 cm^{-1} indicate the A conformation of the hybrid helix. Because no Raman band is found at 835 cm^{-1} , even the DNA moiety is considered to have A-form, in contrast to the suggestion given by S. B. Zimmerman et. al.¹⁷⁾ The fact that the guanine ring breathing band is observed only at 668 cm^{-1} but not at 682 cm^{-1} further points that, not only the RNA moiety, but also the DNA moiety has the C3'-endo guanosine residue instead of the C2'-endo conformer.

DNA hexamer d(CGCGCG) (see Figure 2, lower and Figure 3, lower). The Raman spectrum of this oligomer in a low salt aqueous solution was observed by Thamann et al⁹⁾, and our

Table 2. Guanosine conformation and ring breathing frequency

Ribose Puckering	Glycosidic bond conformation	Guanine ring breathing frequency
C2' endo	anti	682 cm^{-1}
C3' endo	anti	665 cm^{-1}
C3' endo	syn	625 cm^{-1}

present result is in a good agreement with theirs. In both experiments, no strong bands are observed in the $700 - 770 \text{ cm}^{-1}$ region and no shoulder is observed at 795 cm^{-1} . This is understandable because this oligomer has no adenine-thymine base pairs. There are no bands found at 712 cm^{-1} and 813 cm^{-1} assignable to A-form mainchain. Instead, a Raman band appears at 834 cm^{-1} , which is attributable to B-form ribose-phosphate

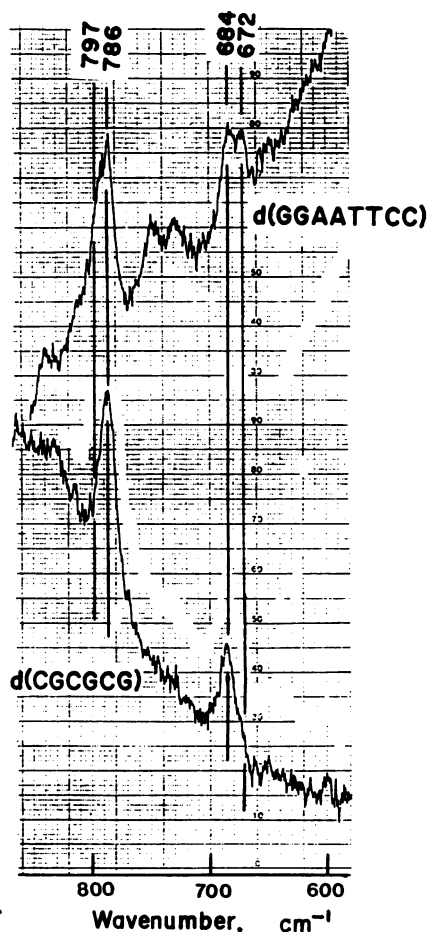


Figure 3. Raman spectra of the same two samples as what are shown in Figure 2, with a higher resolution (4 cm^{-1}) in the $800 - 600 \text{ cm}^{-1}$ spectral region.

mainchain. There is a single strong band at 680 cm^{-1} , assignable to C2'-endo anti guanosine. This fact suggests that all the guanosine residues in this oligomer have C2'-endo anti conformation with no appreciable structural fluctuations.

DNA octamer d(GGAATTCC) (see Figure 2, upper and Figure 3, upper). This oligomer involves a restriction endonuclease (Eco RI) recognition sequence, d(GAATTC). Its mainchain conformation is considered to be primarily of B-type, because a 842 cm^{-1} band is clearly observed here. Because the guanine ring breathing band is found at 680 cm^{-1} , both of the two guanosine residues must be primarily in the C2'-endo anti conformation. This band, however, is found to be appreciably broader and weaker than the corresponding band of d(CGCGCG) (see Figure 3), with a tail in the lower frequency side. This fact may be taken as indicating that there is an appreciable amount of fluctuation of the guanosine conformation towards C3'-endo anti conformation in this oligomer structure. The central portion of this octamer duplex consists of the adenine-thymine base pairs, and Raman bands assignable to this portion are found at 725 cm^{-1} , 750 cm^{-1} , and 795 cm^{-1} (shoulder). It is noticeable that the relative intensity of the 750 cm^{-1} band is greater than that of the 725 cm^{-1} here, whereas the exactly opposite intensity relation was found in the B-form calf thymus DNA (see Figure 1 (g)). This fact suggests that there is an appreciable difference in the nucleoside conformation between the adenine-thymine region of the oligomer in question and the adenine-thymine region (an average) of B-form calf thymus DNA.

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Additional note. After the completion of this work, a recent paper of Brahms et al.¹⁸⁾ has been brought into the authors' attention. They showed that poly (rG-dC) exhibits Raman line at 667 cm^{-1} , which is assignable, in our view, to a C3'-endo anti guanosine residue.

§Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka, Japan

**Faculty of Pharmaceutical Sciences, Osaka University, Suita, Osaka, Japan

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